

## CLINICAL ABORTION AND SEROLOGICAL INVESTIGATION OF *Brucella melitensis*, *Coxiella burnetii* AND *Toxoplasma gondii* IN GOAT'S IN ATAYE BOER NUCLEUS SITE NORTH SHEWA ZONE, ETHIOPIA

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**Abstract.** The present study was conducted to determine the rate of abortion and to investigate seroprevalence of major causes of abortion; *Brucella melitensis*, *Coxiella burnetii* and *Toxoplasma gondii* in goats from January 2016 to December 2018 in Debre Birhan Agricultural Research Center; Ataye Boer Nucleus Site North Shewa, Ethiopia. Data were statistically analyzed by chi-square test using Statistical Package for Social Science (SPSS) software 20.0 version. During three consecutive years of clinical cases study, 513 goats were entered to mating and (19.5%) of the animals aborted. There were a significant difference ( $p= 0.013$ ) in abortion among parities but no difference ( $p>0.05$ ) among goat's breeds. Serum samples were collected from 35 aborted females within six months periods. All samples were screened initially with Rose Bengal plate test (RBPT) for *Brucella melitensis*. All RBPT positive were further tested by i-ELISA. Also, serums were tested for specific antibodies against *Coxiella burnetii* and *Toxoplasma gondii* using i-ELISA. 65.7% and 8.6% of the tested goats were positive for *Coxiella burnetii* and *Toxoplasma gondii* respectively, but neither of them was positive for *B. melitensis*. The present study revealed that *Coxiella burnetii* and *Toxoplasma gondii* were the major causes of abortion in the study site.

**Keywords:** Abortion, Boer goat, *Brucella melitensis*, Clinical, *Coxiella burnetii*, serological investigation, *Toxoplasma gondii*.

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### 1. Introduction

Ethiopia is an agriculturally based country and owns a considerable number of small ruminants, which is estimated to be over 61 million heads of sheep and goats (CSA, 2017). Despite these large small ruminant population sizes, the country failed to optimally utilize these resources as the sector is suffering lower productivity due to various factors in which diseases stand front line (Mohammed *et al.*, 2017). One of the diseases that hamper the productivity of small ruminants is abortion, which has major economic and public health impacts (Mohammed *et al.*, 2017).

Reproductive diseases that result in abortion impose great economical loss in productivity and by product of small ruminants (Mohammed *et al.*, 2017). The common diseases that cause abortion both in goats and sheep in sub-Saharan countries are Brucellosis, Campylobacteriosis, Vibriosis, Chlamydiosis, Foot and Mouth Disease (FMD), Listeriosis, Q-fever, malnutrition, Nairobi Sheep Disease, Rift Valley Fever, Salmonellosis, Trypanosomiasis (OIE, 2018; Ali *et al.*, 2019) and Toxoplasmosis (Peters, 1994; Dubey, 2010). Non-infectious causes of abortion like poisoning,

malnutrition, stress, inherited abortion, vitamin and mineral imbalances brings great loss in production of small ruminant (Peters, 1994).

Several diseases in small ruminants cause abortion or reduced fertility (Szeredi *et al.*, 2006; Mohammed *et al.*, 2017). Toxoplasmosis is zoonotic disease caused by an obligate intracellular parasite known as *T. gondii* (Dubey, 2010). It is the most prevalent parasitic infections in human and veterinary medicine and has negative impacts on public health and animal production. *T. gondii* is believed to be the most triumphant parasitic pathogen in large scale (Dubey, 2010). From wide range of farm animals, sheep and goats are more commonly infected with *T. gondii* than cattle and chicken. This parasite causes abortion and neonatal death in major monetary losses to sheep, goat and pig farming (Tenter, 2000; Jithendran, 2004). This is more serious especially when primary infection occurs during pregnancy (Radostits *et al.*, 2006). During the past decades, *Toxoplasmosis* was reported in different parts of Ethiopia; 35% in Debre Birhan and surrounding areas (Demissie and Tilahun, 2002), 19.7% in East and West Shewa Zones, Oromia Regional State (Zewdu *et al.*, 2013), 15.1% in sheep and goats slaughtered for human consumption in Central Ethiopia (Endrias and Daniel, 2014), 70.83% in Menz and Horo areas (Gebretensay *et al.*, 2019) and 10.25% in small ruminant in Abergele and Ziquala, Amhara Region (Bahiru *et al.*, 2020).

Q-fever is a zoonotic disease caused by *Coxiella burnetii*. Livestock (cattle, sheep, camels and goats) are the main reservoirs of infection to humans (Kaabia and Letaief, 2009; Angelakis and Raoult, 2010). It is also known as an occupational disease of veterinarians, farmers and abattoir workers (De Rooij *et al.*, 2012). *Coxiella burnetii*, the causative agent has been isolated from ticks. Q-fever is frequently misdiagnosed by physicians (Kaabia and Letaief, 2009). It is endemic, both in livestock and humans in North and Sub-Saharan Africa (Schelling *et al.*, 2003; Steinmann *et al.*, 2005; Mazyad and Hafez, 2007). In Ethiopia, the existence of antibody against *C. burnetii* was reported in goats and sheep slaughtered at Addis Ababa abattoir, and it's peri-urban zone (Philip *et al.*, 1966; Gebremedhin *et al.*, 2014). Also seroprevalence of *T.gondii* in small ruminant was reported in Abergelle and Zequala Amhara Region (Bahiru *et al.*, 2020). The diagnosis of *Coxiella burnetii* infection in animals has a great importance to identify the infected flocks and to determine the risk of disease transmission to humans (OIE, 2015; Ullah *et al.*, 2018).

Brucellosis is an animal's disease, especially livestock (cattle, goats, sheep, camels and pigs), but also wild animals (Awah-Ndukum *et al.*, 2018). It is caused by *Brucella spp.* In livestock, it is primarily a reproductive disease characterized by late abortion, retained fetal membranes, Orchitis and impaired fertility (Zinsstag *et al.*, 2011). *B. melitensis* is considered to have the highest zoonotic potential, followed by *B. abortus*, and *B. suis* (Radostits *et al.*, 2007). In Ethiopia, serological studies on brucellosis have been carried out in small ruminant; 9.6% in Southeast Pastoral Livestock (Balako *et al.*, 2013), 1.56% in Yabello districts (Debassa *et al.*, 2013), zero serorevalence in Menz and Horo areas (Gebretensay *et al.*, 2019) and in Abergele and Ziquala, Amhara Region (Bahiru *et al.*, 2020).

During the *peri-partum* period, the massive multiplication of *C. burnetii* and *Brucella melitensis* occurs within trophoblast cells, causing necrotic suppurative placentitis, which ultimately leads to pregnancy failure in the form of abortion, stillbirth, premature birth of weak offspring (Sanchez *et al.*, 2006; Arserim *et al.*, 2011). There were frequent challenges of abortion, stillbirth, retained placenta, premature birth of weak kids in different goat breeds in Ataye Boer Nucleus Site; Debre Birhan

Agricultural Research Centre, Ethiopia (Alemnew *et al.*, 2020a). Also full-term kids were also weak, with low body weight and high mortality (Alemnew *et al.*, 2020b). Therefore the aims of the present study were to determine the rate of abortion and to estimate the seroprevalence of *B. melitensis*, *Coxiella burnetii* and *Toxoplasma gondii* in the study flocks kept in Ataye Boer Nucleus site, North Shewa, Ethiopia.

## 2. Materials and methods

### *Description of study area*

A longitudinal clinical case study on abortion in different goat breeds was conducted in Debre Birhan Agricultural Research Center, *Ataye* Boer goat Nucleus Site from June 2015 to September 2019. The site is found 5km far from *Ataye* town, Eastern Amhara Regional state of Ethiopia and 250 km from Addis Ababa. *Ataye's* climate is classified as tropical. At an average temperature of 25.4 °C, June is the hottest month of the year. December has the lowest average temperature of the year which is 18.7 °C. The climate is characterized by bimodal rainfall consisting of the long rainy season (June-September), short rainy season (February-May), and dry season (October-January). In a year, the average rainfall is 1085 mm (Fekadu, 2015).

### *Animal's management*

The genotypes of the goats evaluated were pure Boer, Central Highland Goat, and 50% Boer (pure Boer cross with Central Highland Goat). A total of 513 goats were used to assess the rate of abortion from June 2015 to September 2019. Flocks were reared with two categories of feeding management i.e. intensive and semi-intensive. Pure Boer goats were managed under intensive management system and 50% Boer and Central Highland Goat were managed under semi-intensive system with grazing and a supplement. The supplement includes *adlibitum* grass hay, chopped pasture (Napier grass, *Desmodium species* and vetch) and commercial concentrate supplement based on their body weight.

Animals were treated using anthelmintic drugs that include albendazole, tetraclozash, tetramisole, ivermectin and triclabendazole. The drugs were applied in three rounds per year in October, February and June following manufacturers' recommendations and deworming months were selected based on the epidemiological cycle of targeted parasite groups and the laboratory findings. Also animals in on-station were sprayed against ectoparasites (ticks, mites, fleas and lice) by using diazinon (60%) and amitrazine (12.5%) and regular vigilance was performed to ensure feeding, herd health care, proper breeding, and cleanliness of the farm (Alemnew *et al.*, 2020a). They were also vaccinated against major infectious diseases which include ovine pasteurellosis (two times per year on October and April), sheep and goat pox (one per year on May), peste des petitis ruminants' /PPR/ small ruminant plague (one per year on September) and contagious caprine pleura pneumonia (one per year on September). Regular vigilance was performed to ensure feeding, herd health care, proper breeding, and cleanliness of the farm.

### *Study design and sample collection*

Abortions were diagnosed up on clinical and post-mortem examination. Aborted animals were grouped based on breeds (Pure Boer, 50% Boer and Central Highland Goat) and round of parity (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and > = 5<sup>th</sup> Days).

Blood samples were collected directly from the jugular vein using sterile vacutainers, about 5 ml of blood was collected. Each sample was labeled using codes describing the specific animal. The tubes were kept overnight at a room temperature to allow clotting. Next morning the serums were separated from blood and collected into 1.8 ml cry vial and were preserved at -20°C until they were processed.

All samples were initially tested by Rose Bengal Test (RBPT), by using 30 µl of serum and 30 µl of antigen (Rose Bengal stained *B. abortus* antigen obtained from BIO-RAD, Marnes-la-Coquette, France) it was mixed and rotated on a glass plate for 4 minutes. Samples with no visible agglutination were recorded as negative, while those showing agglutination were considered positive. For further analysis, all RBPT-positive samples were tested by using i-ELISA kits for *Brucella melitensis*. ELISA kit was obtained from ID Screen® Brucellosis Serum Indirect Multi-species (ID Vet innovative diagnostic Grabels, France). The sensitivity and specificity of the ELISA test kit as provided by the manufacturer were 100% and 99.74%, respectively. The tests were performed according to manufacturer's instructions (OIE, 2009).

All samples were tested against specific antibodies to *C. burnetii* using indirect enzyme-linked immunosorbent assay (I-ELISA). The test use microtiter plates pre-coated with the *C. burnetii* phase I and II strains (ID Screen® Q Fever Indirect Multi-Species, IDvet®). Positive and negative control samples were included in each plate. The sensitivity and specificity of the ELISA test kit as provided by the manufacturer was 99% and 98%, respectively. The test was conducted according to the manufacturer protocol.

Also the samples were tested by using indirect ELISA test using ID Screen Toxoplasmosis Indirect Multispecies manufactured by ID vet, France with wells coated with P30 *T. gondii* antigen to detect the anti-Toxoplasma antibody. The sensitivity and specificity of the ELISA test kit as provided by the manufacturer was 100%. The samples were analyzed in Ethiopian Institute of Agricultural Research; National Agricultural Biotechnology Research Centre.

#### ***Data management and statistical analysis***

Collected data were recorded in MS excel work sheet, after they were checked manually for obvious inconsistencies, recording errors or missing data. Data were analyzed using IBM SPSS V.20 software version. Descriptive statistics were used to determine the rate of abortion and sero-prevalence of *Brucella melitensis*, *C. burnetii* and *T. gondii* in goats. Univariable logistic regression analyses (LR) was also used to measure the level of association between the possible associated risk factors and clinical abortion. A significance level ( $p < 0.05$ ) and confidence level (95%) was set to determine the presence or absence of statistically significant difference between the given parameters.

### **3. Results**

During the study periods 513 goats were assigned for mating, 100 goats (19.5%) aborted. Higher rate of abortion were recorded in the second parturition with 50%, Boer goats with rate of 35.1% and 22.8%, respectively (Table 1).

**Table 1.** Abortion rate in different goat breeds and parities at Ataye Boer Goat Nucleus Site North Shewa, Ethiopia

Variables		No. does	No. aborted	Abortion rate (%)
<b>Breed</b>	Pure Boer	104	22	21.2
	50% Boer	197	45	22.8
	CHG*	212	33	15.6
<b>Parities</b>	1 <sup>st</sup>	104	25	24.0
	2 <sup>nd</sup>	77	27	35.1
	3 <sup>rd</sup>	114	9	7.9
	4 <sup>th</sup>	113	14	12.4
	>= 5 <sup>th</sup>	105	25	23.8
<b>Total</b>		513	100	19.5

Note: CHG = Central Highland Goat

Table 2 showed that the association between different risk factors and abortion. There were a significant difference in clinical abortion between 1<sup>st</sup> (28.1%) and >=5<sup>th</sup> (40.3%) parturition ( $p = 0.046$ , OR = 2.5). Even though there was no statistical difference between first and 2<sup>nd</sup> and 4<sup>th</sup> parturition and were about 1.6 and 2 times higher than first party, respectively. There was no statistical difference ( $p > 0.05$ ) in clinical abortion among different goat breeds.

**Table 2.** Univariable logistic regression analyses (LR) of risk factors (breed and round party) and abortion at Ataye Boer Goat Nucleus Site North Shewa, Ethiopia

Risk factors		No. does conceived	No. does aborted (%)	<i>p</i> -value	OR	Confidence interval (95%)
<b>Breeds</b>	Pure Boer*	57	22 (38.6)	-	-	-
	CHG	139	33 (23.7)	.157	.603	.300 – 1.214
	50% Boer	128	45 (35.2)	.637	1.223	.531 – 2.816
<b>Parities</b>	1 <sup>st</sup> *	89	25 (28.1)	-	-	-
	2 <sup>nd</sup>	65	27 (41.5)	.055	1.959	.985 – 3.894
	3 <sup>rd</sup>	57	9 (15.8)	.494	.716	.275 – 1.864
	4 <sup>th</sup>	51	14 (27.5)	.341	1.595	.611 – 4.163
	>= 5 <sup>th</sup>	62	25 (40.3)	.046	2.504	1.015 – 6.175
<b>Total</b>		324	100 (30.9)			

Note: \* Reference category; CHG = Central Highland Goat

In the present study a total of 35 serum samples were collected from aborted goats within six month periods, 23 (65.7%) and 3 (8.6%) animals were positive for *Coxiella burnetii* and *Toxoplasma gondii* antibody, respectively. But, all animals tested were negative for *B. melitensis* (Table 3).

**Table 3.** Seroprevalence of *Brucella melitensis*, *Coxiella burnetii* and *Toxoplasma gondii* in does at Ataye Boer Goat Nucleus Site North Shewa, Ethiopia

Types of Diseases	No. Animal Examined	No. Animal Positive	Apparent seroprevalence (%)
<i>Brucella melitensis</i>	35	0	0.0
<i>Coxiella burnetii</i>	35	23	65.7
<i>Toxoplasma gondii</i>	35	3	8.6
<b>Total</b>	105	26	24.8

#### 4. Discussions

Abortion imposes great economical loss in productivity in small ruminants. Clinical study on the prevalence of abortion in goats had been conducted at different times in various countries. The result of the present study conducted in selected Ataye Boer Nucleus Site Debre Birhan Agricultural Research Centre, Ethiopia, showed an overall rate of clinical abortion in goats of 19.5%. This rate of clinical abortion indicated the wide distribution of abortion caused by infectious and/or non-infectious agents in goats in the study site.

During the past years, clinical analysis of abortion were reported in Ethiopia; Zegeye *et al.* (2014) in Sirinka Agricultural Research Center, Sheep and Goat Breeding, Evaluation and Distribution Site, Bahiru *et al.* (2020) in sheep and Goats of Abergelle and Ziquala Districts of Amhara Region, Northern Ethiopia, Yizengaw *et al.* (2020) in Sirinka Agricultural Research Centre, Amhara Region and Alemnew *et al.* (2020a) in Debre birhan Agricultural Research Center, Sheep and Goat Breeding, Evaluation and Distribution Site, Ethiopia. The current rate of clinical abortion in this study was comparable with 15.8% reported by Hemayatul *et al.* (2015) in Northern Barind Tract and 22% reported by Ahammad *et al.* (2015) in five upazillas of Mymensingh district in Bangladesh. However, the prevalence of abortion in the present study was higher than 1.4% reported by Innocent *et al.* (2015) at the state veterinary hospital maiduguri, Nigeria. The finding in this study was much lower than the findings of Ahmed *et al.* (2008) with 43.7% in Black Bengal goats in Bangladesh. Such inconsistency in the rates of abortion may be due to the variation in the susceptibility of different breeds to diseases causing abortion, management practices, distribution and causing agents, and measures taken to control the diseases.

The parity was related to abortion in present study with a significant difference ( $p < 0.05$ ) between parities, where the 5<sup>th</sup> parity were about 2.5 times more likely to be aborted as compared to the first one. In this study, older animals presented a higher risk to infect with abortion causing agents than younger animals. In this regard, our result was consistent with Klaasen *et al.* (2014) findings, where the age of the animals was the most significant risk factor for seropositivity of *Q-fever*. Similarly, Gebremedhin *et al.* (2014); Tegegne *et al.* (2016) and Berhanu *et al.* (2018) reported a statistical difference for seropositivity *Toxoplasmosis* and the age of animals.

There was no statistical difference ( $p > 0.05$ ) in the rate of abortion among different goat breeds. This might be due to the management type of animals. Our results confirm Berhanu *et al.* (2018) reports, where no statistical differences were observed among different breeds of goats and sero-positivity of *Toxoplasmosis*.

No seroprevalence of *B. melitensis* was observed in this study, which is comparable with the findings of Muma *et al.* (2006) with 0% in livestock-wildlife interface areas of Zambia, Debassa *et al.* (2013) 1.56% in small ruminants in the Yabello district, Ethiopia, Tadesse (2016) 2.7% overall pooled prevalence of brucellosis in goats in Ethiopia, Gebretensay *et al.* (2019) 0% in goats, Bahiru *et al.* (2020) 0% in small ruminant in Abergelle and Ziquala, Ethiopia. However, the sero-prevalence results of the present study was much lower than of the previous reports of Teshale *et al.* (2006) 13.2% from Somali region, Ashenafi *et al.* (2007) 5.8% in the pastoral region of Afar, eastern Ethiopia, Megersa *et al.* (2010) 12.4% from Borena pastoralist, and Balako *et al.* (2013) 9.6% in Southeast Ethiopian pastoral livestock. The difference in

the management system used in the different countries, types of samples and laboratory test, sampling methods and/or absence of infected goat herds may attribute to such variations. In addition, those differences might be due to variations of the study areas. *Brucella* transmission is favored by a more humid climate, which prolongs the survival of the bacteria in the environment (Teklye & Kasali, 1989).

The seroprevalence of 65.7% of *C. burnetii* in this study was comparable with the findings of Balako *et al.* (2013) 54.2% in Southeast Ethiopian pastoral livestock and Gebretensay *et al.* (2019) 68.33% in Menz and Horo areas, Ethiopian. However, the sero-prevalence results of the present study was higher than the previous reports of Nakoune (2004) with 14.3% in Central African Republic, Schelling *et al.* (2003) 7% in nomadic pastoralists and their livestock in Chad, Klaasen *et al.* (2014) 24.2% in Gambia, and Qudrat *et al.* (2019) 15.0% in Punjab, Pakistan. These variations in the prevalence in different geographical areas can be associated with the farm hygienic measures, routine management practices and environmental factors such as vegetation, soil moisture and the presence of infected animals and vectors in the surroundings (Paul *et al.*, 2012; Rizzo *et al.*, 2016). Our study showed a higher sero-prevalence in goats, which suggests that Q fever, had a considerable importance in animal population in the studied area. The highest seroprevalence observed in this study may be due to genetic susceptibility of goats to *C. burnetii*, higher prevalence of tick vectors to goats, grazing management where animals shared common graze and watering points, agent could be transmitted though aerosol and/or prevailing climatic and weather conditions in the study site.

The seroprevalence of 8.6% of *T. gondii* in this study was comparable with the findings of Bahiru *et al.* (2020) who reported 10.25% in small ruminant in Abergele and Ziquala, Ethiopia. However, the seroprevalence of *Toxoplasma gondii* in this study was higher than the report of Kamani (2010) with 4.6% from goats in Nigeria and was much lower than many reports in Ethiopia as 35% by Demissie and Tilahun (2002), 24.1% by Negash *et al.* (2004) using MDAT and 25.9% using ELISA, 74.8% by Teshale *et al.* (2007), 19.7% by Zewdu *et al.* (2013), 15.1% by Endrias and Daniel (2014), 15.5%, by Gebremedhin *et al.* (2014), 55.2% by Tegegne *et al.* (2016), 27.6% by Berhanu *et al.* (2018), and 70.83% by Gebretensay *et al.* (2019) in Menz and Horo areas.

Also, the present finding was lower than other reports as 26.8% in Ghana (Van Der Puije *et al.*, 2000), 31% in Uganda (Bisson *et al.*, 2000), 27.9% in Thailand (Jittapalpong *et al.*, 2005) and 19.3% in Tanzania (Swai and Kaaya 2013), and as well as 59.4% (Barakat *et al.*, 2009) and 44.3% (Shaapan *et al.*, 2010) in Egypt. The wide variation in the seroprevalence of *T. gondii* infection can be due related to difference in sample size, types of study design conducted, agro-ecology, climate, carnivorous density, farm hygienic practices, animal management, type of serological tests used and the cut-off value used (Dubey, 2010; Opsteegh *et al.*, 2010).

## 5. Conclusion

In the current study, abortion was the major constraint of kid loss with 19.5% in goats. The significant risk factor associated with abortion was parity. Serological investigation of antibody against *Coxiella burnetii* and *Toxoplasma gondii* showed that these agents were the major causes of abortion in the study site. Serological survey highlights the importance of clinical herd health management to avoid economic losses in animals.

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